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SPECIES, PURE AND IMPURE[1]

THERE has come about in recent years a profound modification of our conception of a species in that the botanist, at any rate, is compelled to recognize the fact that Nature presents large numbers of successful kinds of plants that reproduce their types either wholly or in high percentages, but which clearly have germinal constitutions of a hybrid character. These forms may legitimately be described and classified as species and they are frequently virile lines of evolution making up groups of individuals that readily maintain themselves in suitable habitats. As assemblages of like individuals, hybrid as to their germ plasm, they present subjects of study that were not differentiated by the earlier naturalists from the populations of species as they viewed them.

The test of a species, in addition to the characters that distinguish it, has always been the evidence that it breeds true to its peculiarities or so nearly true that variations from the type may be passed over in the descriptive writings of the systematist as exceptions of little importance to the mind seeking for order and rebellious of mental disturbance in his efforts to express this order in accounts of faunas and floras over the earth. There are, then, chiefly as the result of genetical studies of the near present, two conceptions of species.

There is the *pure species* breeding true because its germ-plasm in the diploid condition carries two similar sets of factors, each set contributed by one of the parents and each set having the same genetic make up except for those factors responsible for sex and for sex-linked characters. The pure species was in the main the concept of Darwin and the older naturalists, and it was assumed to be representative of species. As viewed by the cytologist, confident that chromosomes carry

[1] Address of the president of the American Society of Naturalists, thirty-ninth annual meeting, Toronto, December 29, 1921.

the factors or genes responsible for inheritance, the pure species owes its characteristics to the fact that parents contribute chromosomes of identical factorial constitution and therefore give to the zygote pairs of homologous chromosomes with the exception that genes which differentiate sex can only be present in single sets. Expressed in the terminology of the geneticist the pure species is homozygous for all genes responsible for the species' characters other than those of sex, and for sex characters the germ-plasm is heterozygous in either the male or female individual at least where animal forms are under consideration. The problems of sex determination from the diploid sporophyte generations of plants are not yet fully solved. Aside from the possibilities of factorial mutations and of mutations due to irregularities of chromosome distribution a pure species must develop gametes identical for all genes other than those of sex, or linked with sex, because the homologous chromosomes during the reductions divisions separate from one another. Some authors would strictly limit the term species and accept that definition of Lotsy (1914), "A species is the total of all individuals of the same hereditary composition, forming but one kind of reproductive cell." I cannot agree with this opinion since the definition calls for what is almost an abstraction in higher animals and plants, the absolutely pure race.

In contrast to the pure species as defined above is the *impure species,* the germ-plasm of which in the diploid condition carries different sets of genes affecting characters other than those associated with sex. With respect to these genes the germ-plasm is heterozygous and through the reduction division there must take place a segregation of genes with the result that the impure species cannot produce a uniform set of gametes, that is, gametes identical in their germinal constitution. If the diploid germ-plasm is heterozygous for one pair of chromosomes other than the sex chromosomes there would be developed through the separation of the different chromosomes of such a pair two classes of gametes of each sex provided that reduction proceeds in a normal manner. If heterozygous for two pairs of chromosomes there would be developed under normal conditions of meiosis four classes of gametes of each sex, and the theoretical possibilities when larger numbers of heterozygous chromosome pairs are present may be calculated by the well known genetical formula (2^n) when $n =$ the number of heterozygous chromosome pairs.

The impure species is therefore clearly hybrid in its genetical constitution but there is this peculiarity in its breeding behavior that it frequently shows little or no evidence of a segregation of contrasting genes. There is in such cases no obvious splitting off of classes through its progeny, but, on the contrary, the impure species breeds true or nearly true to its type. The true breeding of an impure species must be due to the fact that only favored types of gametes are able to produce in conjugation vigorous zygotes capable of successful development. Furthermore, such favored gametes must carry between them those genes which in combination will reproduce the impure heterozygous germinal constitution of the parent stock.

It is well understood from various plant material that the failure of a hybrid to produce a diverse progeny may be due to irregularities at a number of different points in the life history. The death, the sterility, or the failure of maturation of classes of gametes will eliminate the possibilities of development of whole groups of segregates. Even when viable classes of gametes are formed some may leave no progeny because in conjugation they fail to produce zygotes able to develop a succeeding generation. In plants the length of style, or the nature of its tissues, or of its stigma secretions may operate to check or to limit pollen tube growth or the speed of such growth for some classes of pollen grains and at this point in the life history prevent the functioning of pollen tubes carrying particular types of gametes. Pollen and ovule abortion in greater or less degrees is a very common phenomenon and is responsible at times for the elimination of entire classes of gametes. High degrees of seed sterility and the weak germination of seeds express the

failure of certain types of zygotes to develop a succeeding generation. Explanations for all of these conditions may be offered by postulating lethal factors, as suggested by the work on Drosophila, but it is well to understand for plants how various are the ways in which lethal factors may block the course of development and how numerous are the points at which they may operate.

The significance of the impure species and the importance of its place in certain natural groups is not yet appreciated. Curiously the plant most conspicuously brought to the front as one giving rise to new species by mutation has become one of the forms most thoroughly studied as an example of an impure species. I refer of course to the plant *Œnothera Lamarckiana.* Presented by De Vries as the best illustration of his view that pure species at times pass through periods when they actively produce by large saltations new species, the status of *Œnothera Lamarckiana* from the first became a subject for sceptical examination on the part of a body of naturalists who hesitated to accept De Vries' conclusions, and sought for other hypotheses to account for its remarkable behavior. Bateson was the first to suggest that the fifty per cent. or more of pollen sterility in *Lamarckiana* indicated a hybrid constitution. Jeffries pushed this argument with force through comparisons of pollen sterility in *Lamarckiana* with similar conditions in various known hybrids. Workers with Œnothera now generally recognize for most of their material the presence of very high degrees of sterility both gametic, as indicated by bad pollen and abortive ovules, and zygotic, as shown by large proportions of seeds incapable of germination. Renner has recently taken the subject of pollen analysis to a new level by showing that genetic classes of pollen may be distinguished in *Lamarckiana* and in some other œnotheras by differences in the form of the starch grains within the pollen cell and pollen tube. Cytological studies of Gates, Lutz, Stomps, Hance, van Overeem and others have shown that certain of the variants thrown by *Lamarckiana* differ from the parent type in having higher chromosome numbers due to non-disjunction. This non-disjunction seems correlated with a loose association of chromosomes in *Lamarckiana* and other œnotheras that favors irregularities of chromosome distribution at meiosis such as may be expected in hybrid material. Much breeding evidence, chiefly from the work of De Vries, has made it clear that *Lamarckiana* and other œnotheras develop two or more classes of fertile pollen grains which give in various crosses sets of hybrids in pairs, in threes and in fours, good evidence of hybrid behavior. I have shown that with care in the selection of parent stock it is an easy matter to synthesize a large-flowered vigorous hybrid with so many points of resemblance to *Lamarckiana* that it would be difficult to separate in descriptive botany the hybrid from the assemblage of biotypes that pass under the name *Lamarckiana* which, as Heribert-Nilsson has so well brought out, represents a collective species. Furthermore, this hybrid, an impure synthetic species, which I have called *neo-Lamarckiana,* has thrown in each of six generations from selfed seed similar sets of marked variants, and, as pollen parent in appropriate crosses, gives twin hybrids thus paralleling in essentials the characteristic performance of *Lamarckiana.* It is of interest that among the variants from *neo-Lamarckiana* there appear occasional triploid and quadriploid forms comparable to *semi-gigas* and *gigas.* There is no reason to expect that *neo-Lamarckiana* will ever be other than an impure species no matter how close may be the inbreeding and selection to type. It breeds true through only a small proportion of its progeny and we can see nothing that might change this habit so long as the line lives. Finally, against the assumption that *Œnothera Lamarckiana* is a pure species is the fact that the plant is unknown as a wild species and there is strong probability that it arose as a hybrid in England about the middle of the last century.

These are some of the reasons why geneticists rather generally have come to the conclusion that *Œnothera Lamarckiana* is representative of an impure species which reproduces its heterozygous constitution because the viable zygotes produced are for the most part only those resulting from the union of two

different types of gametes, which in combination reproduce the heterozygous *Lamarckiana* complex. Renner in 1914 presented this point of view, after studies on seed sterility in several species of Œnothera, and the conception of impure Œnothera species was rather fully discussed in my paper "The test of a pure species of Œnothera" published in 1915. Thus certain workers with Œnothera were fully aware of the possible significance of gametic and zygotic mortality in relation to problems of Œnothera genetics some years before Morgan and Muller in 1918 discussed the findings of balanced lethals in Drosophila. Renner deserves particular mention as an investigator quick to bring the facts of gametic and zygotic sterility into relation with the peculiarities of Œnothera breeding. As the result of his studies and those of other investigators we have reason to feel confident that most of the œnotheras that have been the subject of experimental study are impure species, that is to say, heterozygous in their genetical constitution.

I am, nevertheless, confident that pure species of Œnothera do exist but it will require much patience in observation, in cytological analysis, and in experimental crossing to establish them. The most promising form in my experience is a line of *Œnothera franciscana,* which has almost perfect pollen and produces seed about ninety per cent. viable. This line I have selfed for eight generations without finding a single departure from the type. The last generation, grown during the past summer, was a culture starting with 1,425 seedlings from seeds experimentally forced to complete germination, a germination percentage of 87.3 per cent. In this large culture 1,373 plants survived the vicissitudes of the season, a loss of only 52 plants mostly as seedlings. This culture was large enough to bring out variants if present in the proportions thrown by *Lamarckiana,* which for some variants is as high as one per cent., but the culture gave no exception to the type. Also, crosses have been made with *biennis, muricata* and *grandiflora* and, when *franciscana* was the pollen parent, the results have been uniform F_1 generations, indicating that the pollen grains of *franciscana* are all alike in genetical constitution. Finally, a cytological study of pollen formation now in progress by my former student R. E. Cleland shows a regular pairing of chromosomes during meiosis in contrast to the loose association of chromosomes characteristic of the same stage in *Lamarckiana* and such other œnotheras as have been studied with the exception of a race of *grandiflora.* Thus the evidence of high fertility, uniform progeny when selfed, uniform F_1 generations when used as the pollen parent, and regularity of chromosome pairing during meiosis all point to the genetic purity of this race of *Œnothera franciscana.* I present this line as the purest Œnothera material known and safer than the race of *grandiflora* that I selected twelve years ago and which satisfied fairly well the tests of a pure species except that it threw occasional weak dwarfs. This isolation of an apparently pure species of Œnothera is a matter of satisfaction and of some importance for the future of genetical studies in this group of plants since in the past we have had no standard material of unquestioned purity with which forms could be mated in tests of cross breeding. My apparently pure race of *Œnothera franciscana* is vigorous, easily grown in cool latitudes, and has a long flowering season, qualities important for experimental work, and I confidently offer it to students of Œnothera as a plant worthy of their attention.

The interpretation of the breeding behavior of *Œnothera Lamarckiana* on the hypothesis of its impure germinal constitution has received important and most substantial support from the investigations of Muller on material of Drosophila which led to his theory of balanced lethals. The condition of balanced lethals results when two different lethals are present, the first in one chromosome and the second in the other chromosome of a pair. Thus each lethal is present in a single dose and the genetical constitution is therefore heterozygous for each lethal but the two lethals are in different chromosomes of a synaptic pair. Since the lethals operate when in double doses close breeding in such a race will result in a succession of generations repeating the

heterozygous genetic formula because the homozygous associations of either lethal block further development. Such a factorial situation would maintain a state of constant heterozygosis, the fixed hybridism of an impure species. The genetical impurity will be passed from generation to generation and in this respect the hybrid will breed true until the relative positions of the lethals are changed by a crossover, or the genetical constitution in these respects is altered by a mutation. A crossover frees at once recessive characters which were suppressed by lethals in homozygous condition and the sudden appearance of such recessives will simulate mutations although in reality they are manifestations of a process of segregation.

The theory of balanced lethals offers such a satisfactory interpretation of the behavior of certain Drosophila material, behavior similar in nature to that of *Œnothera Lamarckiana,* that Muller was quick to suggest the application of his results to Œnothera problems. It should be noted that De Vries as early as 1911 offered a hypothesis essentially similar to the theory of balanced lethals to account for the peculiarities of the double reciprocal crosses between *Œnothera biennis* and *Œnothera muricata,* forms which, on strong evidence from the studies of Renner, we now believe to be impure species. Investigations of my own, published in 1917, on these hybrids and on others support the conclusions that lethals are common in Œnothera material, but I believe that conditions are more complex than indicated by the conclusions of De Vries and Renner. De Vries in recent papers has also made free use of lethals in offering hypotheses to cover certain results of his breeding studies with Œnothera.

Although it is not my purpose to discuss the mutation theory of De Vries it does seem important to examine critically the position of this theory as it is affected by the evidence for the existence of impure species that are held to a behavior of pure breeding or almost pure breeding by lethals which suppress the appearance of segregates. Lethals are not rare in Drosophila and Œnothera material. There is reason to suspect that they are common manifestations of irregularities in the mechanism of the organism of so serious a nature that they interfere with vital processes at some point in the life history, finally bringing the machine to a standstill with death as a result. The workers with Drosophila seem inclined to believe that much of the phenomena simulating mutation in their material is in reality the appearance of characters set free by the breaking of lethal adjustments which held the characters latent. Well known workers have arrived at similar conclusions for Œnothera material and are not content to accept as evidence of mutations the behavior of *Lamarckiana* and some other forms when they throw their marked variants.

An entirely new conception of mutation phenomena has grown up with meaning very different from that of the past. Œnothera material selected by De Vries on the assumption that it illustrated mutation in a pure species proves to be highly impure and in genetical constitution exceedingly complex. Progress in the study of mutations must follow the usual course in genetical research and rest upon intensive studies of particular characters, analyzed and traced through experimental cultures and tests of cross breeding, with the assistance of cytology at critical points in the life history, and with constant attention to phenomena of infertility and sterility. From the later writings of De Vries it would seem that the master recognizes the newer trend. Logically mutations appear to be more likely from hybrid stock than from pure lines since heterogeneity of germinal constitution obviously invites chemical and physical modifications that might lead to the origin of new genes or to such changes in old genes as would result in different expressions of former characteristics. Of particular import is the expectation that lethals most frequently owe their presence to heterozygous conditions since the mixing of diverse germ-plasms seems likely to lead to the breaking down of delicate and vital adjustments in proportions relative to the degrees of protoplasmic confusion, and this means chemical and physical disturbance. The intensive study of specific mutations with its effort at analysis to the last degree is a

very different matter from that care-free attitude of former years which permitted any marked variation not easily interpreted to pass as a mutation. Mutation has become intimately a part of that most fundamental and illusive problem of biology, the origin of variation, and mutations apart from the study of their causation are of secondary interest.

Œnothera material and lines of Drosophila were not the first representatives of impure species to be isolated by the geneticist. The blue Andalusian fowl which cannot be fixed, yellow mice that never have the double dose for yellow, Vilmorin's dwarf wheat which throws talls but fails to produce homozygous dwarfs, single stocks never homozygous for singleness, these and other cases are well known and proven examples of impure species heterozygous in their germinal constitution. Certain of them, as the blue Andalusian fowl, throw two homozygous types, in this case the black and white "wasters." Others produce one viable homozygous type. Some impure species rarely and perhaps never throw homozygous segregates. All agree in this respect that the heterozygote, which breeds true to its proportion of the progeny, can not be fixed by selective inbreeding although as an impure species it reproduces itself with exactness.

We have briefly reviewed conclusions from the intensive study under experimental conditions of lines which genetical investigations have established as representatives of impure species. Some of the material is obviously of the sort that would not hold its own under conditions of open competition in Nature, but much of it has been derived from forms not far removed from wild species. There is a broader aspect of the subject of the hybrid deserving of examination, namely, the study of the possibilities of the impure species as a definite component of faunas and floras.

First of all it is important to bear in mind that if we accept the current theory which places the determination of sex as a function of the reduction or segregation divisions, all unisexual animals are heterozygous for sex factors and for such genes as are responsible for sex-linked characters. For higher animals this means that either the male or female carries in single dose a chromosome which is not paired with an equivalent chromosome. For higher plants we should expect the diploid sporophyte generation to be heterozygous for sex determining chromosomes, a condition for which as yet we have cytological evidence from only one type, the liverwort Sphærocarpos studied by Allen and his students, although there is experimental evidence for this condition in other liverworts, in some unisexual mosses, and in certain seed plants, *e. g.*, Melandrium. The behavior of sex-linked characters may then be believed to follow an orderly system in inheritance except as such linkage is broken or as point mutations appear in sex chromosomes.

But accompanying the sex chromosomes are those groups of chromosomes, the autosomes, responsible for characters not of sex or sex-linked. The unisexual state precludes the possibility of that closest form of inbreeding possible through hermaphroditism and leaves the way open to outbreeding subject only to physiological limitations and to conditions whereby lethals prevent reproduction. That Nature has made extensive use of this encouragement of outbreeding in various degrees cannot be doubted, and this is best illustrated in man, the most mixed and varied of all animals in the assortment of genes carried by the individual. It is impossible to believe that any human is homozygous for the complex of factors responsible for his individuality.

Even when, as in most higher plants, the diploid sporophyte generation is bisexual, there have arisen in many lines of evolution conditions that make for very high degrees of genetic impurity. There was a time in the history of botany when workers, following the lead of Darwin, devoted themselves to the study of devices to secure cross-pollination and many and remarkable are the arrangements described to encourage outbreeding. Volumes have been written on this subject and the facts in general are freely admitted. In wind-pollinated forms there is even greater opportunity for promiscuous pollination unless the shedding of pollen takes place at such a time that stigmas are dusted and the ovules self fertilized before outside pollen has had an opportunity to reach

the pistil. Perhaps the best examples of wind pollinated types very freely open to outside pollination are the numerous races and forms that make up the collective species *Zea Mays.* The studies of East and Jones, Emerson, Shull, G. N. Collins and others, extending over many years, show conclusively that corn is usually a hybrid composite with so many characters represented by genes in single doses that purification of material by selective inbreeding is a matter of much time and patience. There could hardly be a greater contrast in genetical behavior than that between lines of wheat which, because they rarely outcross, breed very true, and races of corn that can only be kept reasonably true by constant watchfulness, practiced selection, and a never-ending elimination of products departing from the types.

Self-sterility and the production of weakened generations following inbreeding, as factors leading to the establishment of impure species, have not as yet received recognition proportionate to their importance. Genetical studies seem likely to show that there are large groups of bisexual plants the individuals of which are either infertile when selfed or produce progenies in successive generations distinctly inferior in vigor to the wild types. In such material the species represented in Nature must be very largely, if not wholly, made up of individuals cross-bred and genetically impure. It is significant that these conditions should have been found in that most successful assemblage, the Compositæ, frequently cited as the climax group of plant evolution. The recent studies of Stout on chicory have shown the extensive presence of self-sterility, and that the wild populations must consist chiefly of outbred and probably heterozygous individuals. Investigations of J. L. Collins on Crepis indicate that species of this genus are impure since progeny from selfed lines show marked deterioration from the wild stock as segregation proceeds and forms approaching purity of germinal constitution are isolated. Crepis seems likely to prove an assemblage of impure species similar to that assemblage of impure races called *Zea Mays,* and will probably show the same parallelism of behavior in reduced vigor and the production of abnormal types as inbred lines are separated from the wild population. The interpretation for Crepis is likely to be that of East and Jones for maize, namely, that inbreeding gives deleterious results through the segregation of types with fewer genes for characters associated with physiological vigor of expression. These studies are tending towards conclusions well established for many cultivated fruits, as apples, pears, plums, cherries, etc., where self-sterility among the varieties proves to be the rule and cross-pollination is necessary for sexual reproduction through impure lines. It is hardly possible that chicories and species of Crepis are outstanding exceptions to conditions in the Compositæ and we may safely predict that studies in this immense assemblage will reveal wide-spread the presence of impure species. Self-sterile lines among the grasses have also been reported, *e. g., Lolium perrene.*

There is another type of impure species not represented in the animal kingdom but common in certain groups of plants and therefore of particular interest to the botanist. This is the hybrid which perpetuates itself by vegetative means and thus establishes populations in the wild when its characters are favorable to survival under the scrutiny of natural selection. The well known principle of hybrid vigor, or heterosis, may in itself be expected to give to such hybrids marked advantage. These impure species hold true to their characters through asexual reproduction although by their seed they may produce a large variety of segregates. This principle of the maintainance of a hybrid by vegetative reproduction is applied in agriculture when selected lines of potatoes are propagated from slices of the tubers and strawberries from plants developed by the runners, and in fruit culture by the grafting of choice hybrid varieties.

There have been two notable systematic studies in America on groups of wild species in which hybrids are found well established as impure species. Brainerd's investigations on the violets and blackberries show the possibilities of critical studies on the status of species, making use of the experimental garden and basing results on genetical analyses. Favored

hybrid blackberries, spreading readily by prostrate branches that root at the tip, may easily establish themselves in extensive growths. In a recent classification of the blackberries of New England Brainerd and Peiterson isolate 23 hybrid species of the 12 primary species that are recognized, and they give an additional list of 32 suspected hybrids. Violets do not spread so prolifically as brambles but there are a number of hybrids known which maintain themselves in Nature by vegetative growths. Other groups of plants readily propagating from stems are likely to show similar proportions of impure species as they are more thoroughly studied.

With the data before us on the widespread occurrence in Nature of impure species we wonder what will be the reaction of systematic botany. It will be impossible for the manuals to include the many hundreds of lines which the geneticist may isolate as impure species although they may be definite units of floras. There will be little satisfaction in attempts to identify in the field races which can only be established by experimental studies of the garden. Are these impure species to be grouped for convenience as collective species regardless of their true positions and relationships? Truly the paths of the systematist and ecologist have not been made easier by the progress of genetics.

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THE TREND OF EARTH HISTORY[1]

II

Through the millions of years represented by the Tertiary period the mammals differentiated slowly along the conventional lines which had been previously marked out in large measure by the reptiles. Some became adapted to life on the dry plains, others in the forested river flats, others in the high mountains, the tree-tops and the tropical jungles. A few of them learned to fly more or less successfully, some burrowed under ground and still others became aquatic. In a general way they did what the various kinds of reptiles had done before them in the Mesozoic era, but, on the whole, they seem to have done it better.

Finally, about the end of the Tertiary period or later, the next great advance was made by the genus *Homo*—an offshoot of one of the most insignificant groups of mammals. In consequence of this achievement, the entire group has been dignified with the name of *Primates*. From this offshoot so many surprising things have developed that it is hard to say which one was fundamental. Undoubtedly, one of the first new habits of the human genus was the use of tools. We may reasonably suppose that only one of the less specialized types of mammals, a creature possessing flexible fingers and hence the power to grasp a stone or a club in the hand, could acquire such ability. Possibly it was this initial power that gave the first impetus to the higher progress of the pre-human stock. Be that as it may, the progress of the human race seems to have depended largely on the ability to invent and use other things, such as fur-covered skins for clothing, the spear and bow-and-arrow for the chase, the fish hook, the needle, the potter's wheel and so on through the long list of human contrivances. As Bergson has remarked, each human tool and machine serves as a new and additional bodily organ and so multiplies our functional activities to a wonderful degree. The development of higher intelligence went on side by side with this multiplication of inventions, doubtless, on the one hand, being stimulated by it and, on the other, making possible its continuation.

Looking back over the great contributions which the various animal groups have devised and elaborated in the vast stretches of geologic time, and omitting only that of the human race—which is too new to be impartially judged—it will be observed that, although each of these innovations has brought temporary success and domination to its holders, it has never been able to insure the permanency of the exalted position so attained. Experimentation seems to be nature's endless pastime. Her appetite for it is insatiable; and, no matter how interesting the results of the trials already made, there are always more to come. As John Burroughs once said, "Nature hits the mark, because she shoots in all directions."

In that part of the history of man which is sufficiently well known, we perceive a series of